Validation of Bioassays for Vaccines

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Assaying Potency of Novel Vaccines

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Outline

- Why validate?
- Assay characteristics ➔ Model
- Assay validation parameters
- Reportable value ➔ Power of averaging
- Acceptance criteria

Why Validate?

- Regulatory Expectations
- Measurement is the foundation on which research decisions rest
  - Don't think of validation as pass/fail
  - Use the validation results to inform your routine choices

➔ replication – informed averaging

When ?

- As soon as you start making decisions using the data
- After the optimal operating conditions have been established in assay development – stable operating conditions
  - Driving intra-assay factors
  - Driving Inter-assay factors
- Continuous assessment

➔Early and continuously thereafter

Assumptions

- Measure is a biologic response (activity not mass)

  ➔ highly variable

- Continuous response – or at least convertible
- Simplistic Layout

Lot 1 … Lot j	=> Inter-lot (j)	➔ S_L
Run 1 … Run r	=> Inter-assay (r)	➔ S_b
Prep1 … Prep k	=> Dosage Unit (k)	➔ S_{DU}
Rep1 … Rep n	=> Intra-assay (n)	➔ S_w

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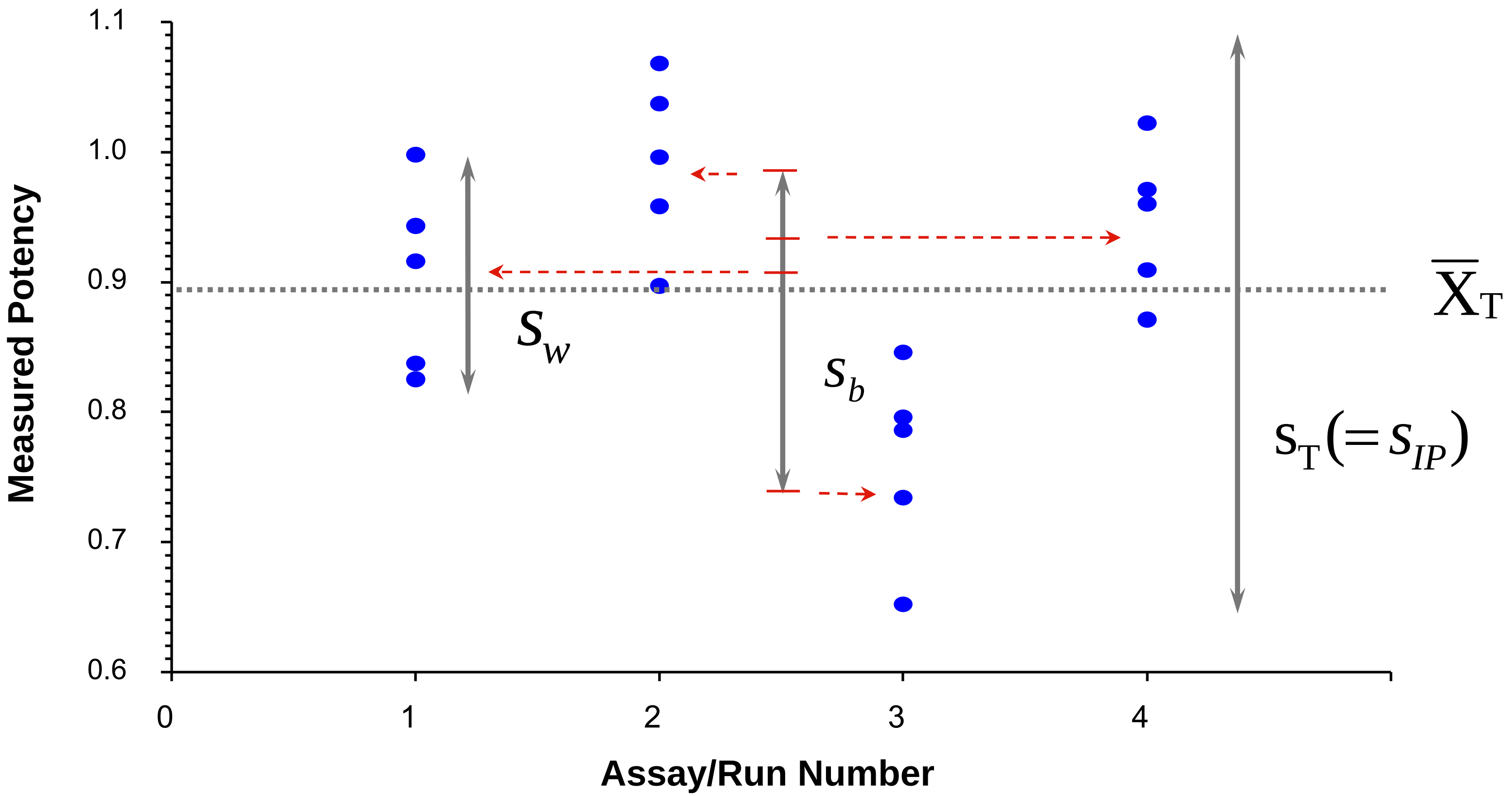
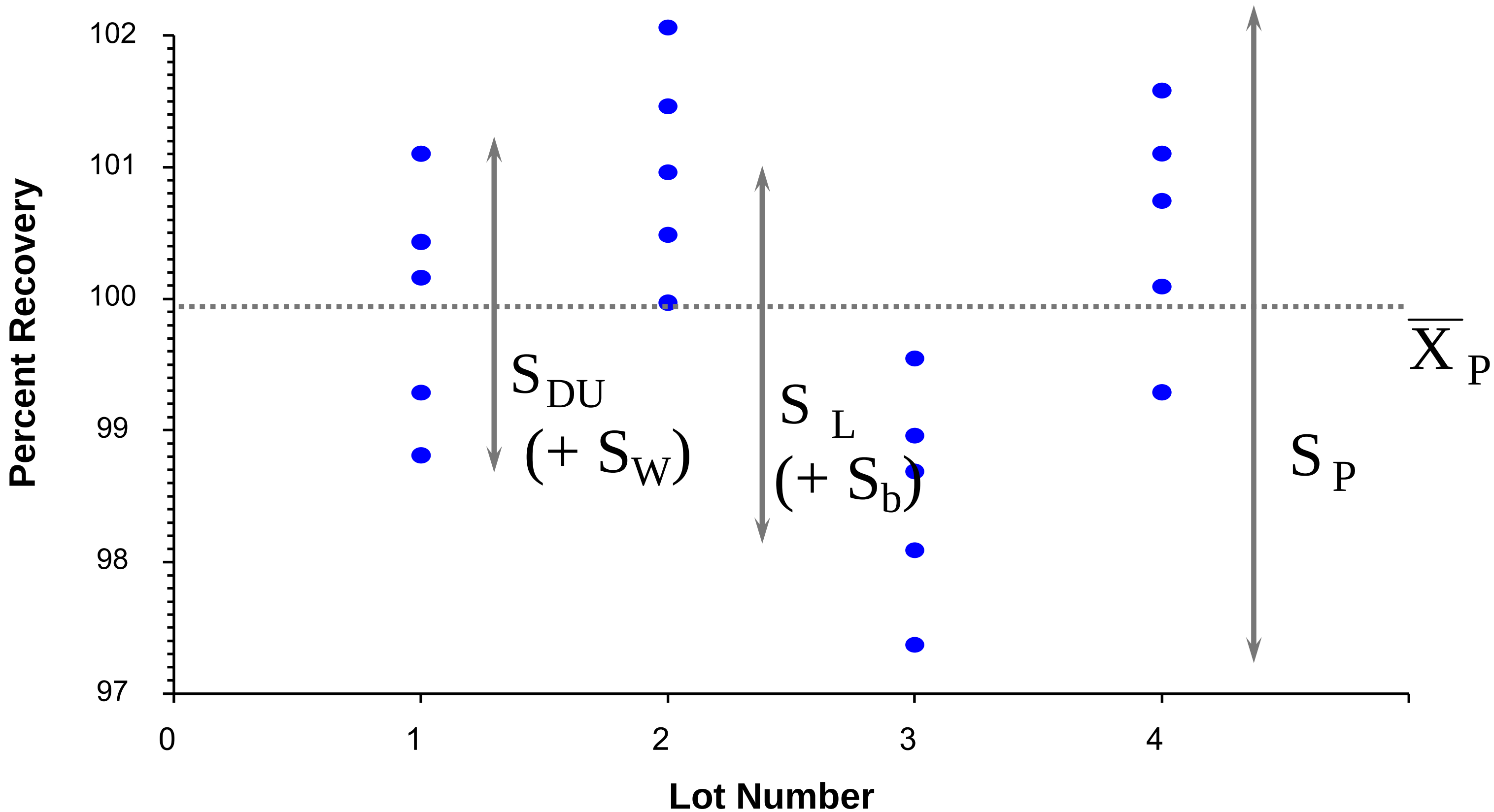
Model of the Assay



Broader Model – Process (& Assay)



Assay Validation Parameters

- Relative "accuracy"/linearity
  - Dilution effect
  - Forced degradation

$$\left.\begin{array}{l}\text{Dilution effect}\\ \text{Forced degradation}\end{array}\right\} \overline{X}_A + \overline{X}_P$$

- Precision
  - Robustness – intra-assay factors
  - Ruggedness – inter-assay factors
  - **Reproducibility** – random draw routine control - $S_T(\rightarrow S_{RV})$
- Others are variations on accuracy or precision
  - Limit of detection, range, interference

Validation Design - Precision

- Replication pattern targeted toward primary noise sources
  - Intra-run noise
  - Inter-run noise
    - Run is independent preparation of reagents, test, and standards
    - Don't short change the number of runs (≥ 6)
- Choice of levels dictated by range of product potency
- Avoid pass/fail mentality – worst case, not best

Assay Capability – Getting the Numbers

- Estimates based on standard deviations of the individual replicates and the run averages.

$$\left.\begin{aligned} s_w &= Avg(s_{Run1}, s_{Run2}, \ldots) \\ s_b &= s_{\overline{X}} - (s_W / n) \end{aligned}\right\} \text{ANOVA}$$

- 3 Rules:
  - Noise is cumulative ➔ $S_{All}^2 = S_1^2 + S_2^2 + \cdots$
  - Averaging improves precision – **predictably** ➔ $S_{Avg}^2 = \frac{S_{y_i}^2}{n}$
  - Mean ± 3s bracket the results

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Assay Capability - Using the Information

- Reportable Value
  - What constitutes an assay?

- Sources of Noise (Propagation of Errors)
  - Control by averaging

$$S_{RV}^2 = \frac{S_{DU}^2}{k} + \frac{S_W^2}{n} + S_b^2$$

$$\boxed{\begin{array}{c}\text{Average of c composites:} \\ S_{RV}^2 = \dfrac{S_{DU}^2}{c*k} + \dfrac{S_W^2}{c*n} + S_b^2\end{array}} \Bigg/ r$$

In viral/bioassay average over r runs

Establishing a Reportable Value

- How do you define the rv?

  ➔ Impact, Criteria, Cost

- Impact of the sample allocation.

Suppose, $\left.\begin{array}{l} s_w = 17\% \\ s_b = 25\% \end{array}\right\}$ $S_{RV} = \sqrt{\dfrac{S_w^2}{n \cdot r} + \dfrac{S_b^2}{r}}$

n	Runs (r) 1	6	12
1	30%	12%	9%
2	28%	11%	8%
3	27%	11%	8%

Acceptance Criteria

- Acceptance Criteria dictated by use of the assay
  - Define use by a range or specification limits
- Adjust the replication so that,

$$6\sqrt{S_P^2 + S_{RV}^2} \le Range$$

- If our desired range is 50% to 150%

$$\rightarrow \quad S_{RV} \le 17\%$$

How much replication is too much?

- Replication vs Method Improvement
  - Partly driven by **$**
- Capability of the Art ?

	S_T	Range
• Small Molecule (HPLC)	<5%	±15%
• SM in matrix (GC/Mass Spec)	15%	±45%
• Bigger Molecule (Immunoassay)	20%	±60%
• BM activity (Bioassay)	50%	±150%
• Viral Assay		

But these can be easily reduced by 60% just by judicious averaging

So, what level of S_{RV} do I target?

Pitfalls

- Limited data has risks
  - Some risks are controlled by choice of multipliers
  - Look for ways to update and expand information

    ➔ Follow-up (continuous assessment)

    Stability studies, Control samples, Scale-up
- The curse of the validation experiment
  - We tend to reward pass/fail rather than good information
- You will likely need to work in log scale

Data Driven Release Specifications

Data Driven Expiry Specifications

ES = Process/Assay Mean ± Drift – Degradation ± Uncertainties

$$L/UES = \bar{X} \pm S_L + \beta * T \pm 3 * \overbrace{\sqrt{S_L^2 + T^2 * S_\beta^2 + S_{RV}^2}}$$

Then ask:
Are these specs pharmacologically sound?
Are they close to what agencies are asking for?

Recommendations

- Define carefully what values should be held up to the specification – reportable value.
  - Do NOT expect individual values to meet those same specs.
  - Paradox of individuals – disincentive to collect more data.
- Validation is a continuous process
  - Utilize all of your information